

OPINION

by

Assoc. Prof. Vesela Ivanova Georgieva, MD,

Department of Microbiological Factors

**Directorate of Analytical and Laboratory Activities, National Center for Public Health
and Analyses, Sofia**

Subject: procedure for awarding the educational and scientific degree "Doctor" in professional field 7.1. Medicine, scientific specialty "Hygiene"

Topic of the dissertation: *"Hygienic and analytical aspects in the study of the seasonal dynamics of microcystins in surface waters for drinking purposes"*.

PhD student in independent training: Maria Angelova Mitreva, Chief Assistant Professor in the Department of Chemical Factors, Directorate of Analytical and Laboratory Activities, National Center for Public Health and Analyses, Sofia

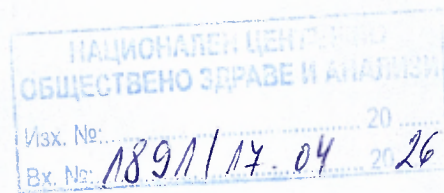
By order No. RD-146/18.03.2026 of the Director of National Center for Public Health and Analyses, I was elected as an internal member of the scientific jury.

The doctoral student has submitted all necessary documents for the procedure for acquiring the educational and scientific degree "Doctor" in accordance with the requirements of the Law on the Development of the Academic Staff in Republic of Bulgaria (LDASRB) and the Regulations for the Implementation of the LDASRB of the National Center for Public Health and Analyses

Brief biographical data of the doctoral student

Chief Assistant Professor Maria Angelova Mitreva received a Master's degree in Medical and Pharmacological Biophysical Chemistry at Sofia University "St. Kliment Ohridski" in 2013, and in February 2017 she was awarded the educational and scientific degree "Doctor of Chemistry". Since March 2017, she has been an assistant, and since October 2019 - chief assistant at the "Analytical and Laboratory Activities" Directorate at the National Center for Public Health and Public Health.

Her professional activities are related to chromatographic methods for the analysis of pollutants in water; analysis of environmental samples, food and beverages; spectral methods of analysis; methods for separation and concentration; determination of forms of chemical elements. Maria Mitreva participates in scientific projects at the Scientific Research Fund under the Ministry of Education, and is also the head of one of them. Participates in scientific topics of the NCPHA.



The dissertation submitted to me for opinion is in accordance with the procedure for acquiring the educational and scientific degree of "Doctor". The chosen topic of the dissertation is relevant and significant from a scientific and applied-practical point of view, due to the growing need to provide modern analytical data on the presence of microcystins in water sources supplying drinking water to settlements.

Cyanobacteria, also known as blue-green algae, are one of the most ancient groups of prokaryotic, autotrophic microorganisms. They use a variety of metabolic strategies to survive and adapt to different environmental conditions.

As a result of the increased anthropogenic pressure on all ecosystems, including aquatic ones, the processes of eutrophication of water bodies are accelerating, which leads to blooms of phytoplankton inhabiting water bodies. Under favorable physicochemical conditions of the water, such as temperature, light, pH, presence of limiting nutrients (nitrogen / phosphorus) in sufficient concentrations, cyanobacterial blooms can be observed, which, along with deterioration of the organoleptic properties and increased turbidity of the water, could also be a source of cyanotoxins, dangerous to human health.

The current requirements for drinking water quality in Europe are set out in Directive (EU) 2020/2184 of 16.12.2020 on the quality of water intended for human consumption. Among the new pollutants that will be mandatory for monitoring from 2026 and for which the risk of contamination of water bodies should be assessed, is microcystin-LR. It is a representative of the microcystins, which are a group of cyanotoxins produced by blue-green algae.

The text is presented on 141 standard pages and is illustrated with 31 figures and 24 tables. The bibliography includes 153 literary sources, of which 12 are in Cyrillic. There are 3 publications related to the dissertation.

The introduction and literature review demonstrate the doctoral student's very good awareness of the problem being developed and correspond to the title of the dissertation.

The literature review covers detailed scientific information about *cyanotoxins*, such as:

- Characteristics of cyanotoxins
- Classification of cyanotoxins
- Cyanobacterial blooms and negative impact on human health

- Drinking water sources and recreational water sources for which there are scientific data and publications commenting on cyanobacterial blooms in them
- Influence of environmental factors on the formation of toxins
- Globality of the problem and its importance for Bulgaria
- Methods used for the determination of microcystins

Knowledge of information in this area has been demonstrated, which has been used to form the goal and objectives, select analysis methods and interpret the results obtained.

The goal of the project is precisely and clearly formulated - development, validation and application of analytical methods for determining microcystins in surface waters for drinking purposes, as well as characterization of the condition of selected dams in the area of the city of Sofia and the district by studying the content of these toxins and assessing the dependence between their release and environmental factors.

- To achieve the set goal, *four main tasks* have been formulated:
 - Development and validation of analytical methods for qualitative and quantitative determination of microcystins in water samples;
 - To determine the content of microcystins in surface waters for drinking purposes.
 - To monitor the dynamics of the species composition of cyanobacteria and the presence of microcystins in selected dams in the area of the city of Sofia and the region.
 - To assess the influence of environmental factors on the production of microcystins in real conditions.

In the "Materials and Methods" section, the doctoral student has clearly and in detail presented the equipment used and the methods applied.

The "Results and Discussion" section of the dissertation presents summarized results from studies conducted during the period June–October 2023, 2024 and 2025, aimed at studying the seasonal dynamics of microcystin–LR and its homologues –RR and –YR in water samples from main water sources for drinking water supply in the area of the city of Sofia and the region: Iskar Dam and Kokalyane Dam, Studena Dam and Krasava Dam.

A total of 99 water samples were analyzed, taken from different points and depths, and the results are presented in separate points for the respective water bodies. In addition to microcystins, the samples were analyzed for the content of total phosphorus and total nitrogen, and the phytoplankton communities inhabiting the studied water bodies were also

characterized. The statistical processing of the results was applied through correlation and regression analysis (Pearson) at a significance level of $p < 0.05$.

Three points are surveyed at the **Iskar Dam** - a water intake tower, a Stork's Nest, and a sailing base.

- The results of the conducted research show an extremely low level of toxins in the area of the *water intake tower of Iskar Dam* during the period 2023–2024. In most analyzed samples, the values are below the limit of quantification, with only two out of a total of twenty analyzed samples (both from July 2023) detect trace amounts of microcystin–LR equivalent ($\sim 0.2 \mu\text{g/L}$) – a compound with a similar spectrum, but with a different retention time compared to the target analytes. Correlation analysis shows a moderate positive relationship between microcystins and total nitrogen in 2023, while the relationship between microcystins and total phosphorus is less pronounced. The obtained relationships are not statistically significant ($p > 0.05$), but show a relationship to an increase in the concentration of microcystins at higher nutrient values. In 2024, all measured concentrations of microcystins are below the limit of quantification and are calculated as LOQ/2, therefore there is no variation in the indicator and a correlation analysis between microcystins and nutrients cannot be performed.
- Compared to the area of the water intake tower, *the Stork's Nest point* is characterized by higher variability of nutrients and more frequent registration of trace amounts of toxins, which defines it as a more sensitive area. However, no toxic cyanobacterial blooms were observed and the ecosystem maintained a stable oligo-mesotrophic status. Correlation analysis showed weak relationships between microcystin concentrations and nutrients, with the obtained correlation coefficients being low and not statistically significant ($p > 0.05$).
- The results of the studies show low concentrations of microcystins in the *area of the sailing base* during the period 2023–2024. Compared to the other studied points of Iskar Dam, the sailing base is distinguished by moderate nutrient enrichment and the least pronounced toxin production, which defines it as a hydrodynamically more active zone with a low risk of contamination with cyanotoxins. The correlation analysis shows a positive relationship

between microcystin concentrations and nutrients in 2023, without statistical significance ($p > 0.05$) probably due to the limited number of observations, and in 2024, due to the lack of variation in microcystin values, an analysis could not be performed.

The results of the studies show low concentrations of microcystins in the **Kokalyane Dam** during the period 2023–2024, with the values below the limit of quantification in most samples. In 2023, trace amounts of microcystin-LR equivalent were detected in individual samples (July and August), while in 2024, no toxins were detected. The measured concentrations are significantly below the reference value for drinking water and do not pose a risk to the water supply. The observed nutrient concentrations show moderate variation, with the detected trace amounts of microcystins coinciding with the highest values of total nitrogen, confirming the trend for a relationship between nitrogen enrichment and the initiation of toxin release. The correlation analysis shows a positive, but not statistically significant, relationship between microcystins and nutrients in 2023, and in 2024 the lack of variation in microcystin concentrations does not allow for a correlation analysis.

The complex analysis of the results from the studied points of Iskar Dam (water intake tower, Stork's Nest and sailing base) and Kokalyane dam shows that despite local variations in nutrient enrichment, the ecosystem retains its stability and no risk to the quality of drinking water supply is observed.

Regarding the **Studena Dam**, the results of this study indicate the presence of microcystins in a limited number of samples. In June and July 2023, trace amounts of microcystins (LR-equivalents) were detected in samples from the Water intake tower of Studena Reservoir and the Studena Reservoir shore site. The most significant results were recorded in October 2023, when a total microcystin content of 1.2 $\mu\text{g/L}$ was found in the water intake tower; MC-LR is 0.38 $\mu\text{g/L}$ (~32% of the total content); and even higher concentrations, approaching the regulatory value, were found in a surface bloom near the dam wall. The correlation analysis for the period 2023–2025 does not show a statistically significant relationship between microcystins and nutrients ($p > 0.05$), indicating that toxin production is not directly determined by nitrogen and phosphorus levels, but is the result of a complex interaction of environmental factors. Overall, the Studena Dam can be defined as a water source with a stable ecological state, but with a demonstrated potential for episodic production of cyanotoxins, which requires increased monitoring, especially in the late summer and early autumn periods.

Regarding the **Krasava Dam**, the results of the conducted studies show that the reservoir is characterized by a stable oligo-mesotrophic status and low concentrations of microcystins during the period 2023–2025. The correlation analysis shows weak to moderate dependencies between microcystin concentrations and nutrients.

The comparative analysis shows that the Studena reservoir has the highest potential for episodic production of microcystins, including values approaching or exceeding the reference limit for drinking water (1 µg/L), while the Iskar reservoir and the Krasava reservoir are characterized by a lower toxicological risk and higher ecological sustainability. The studied Bulgarian drinking water reservoirs Iskar, Studena and Krasava are characterized by a good ecological status and a low to moderate risk of contamination with microcystins, as the main factor for the occurrence of toxic blooms is not the amount of nutrients, but the complex impact of physical, chemical and biological processes.

In all tests performed, the analysis of variation showed good agreement between the results obtained by ELISA and HPLC, which confirm the reliability of the applied analytical methods.

Based on the scientific work provided to me, I would summarize that it represents a comprehensive scientific study on a current topic and contains scientific contributions regarding the content of microcystins in surface waters for drinking purposes. The goal has been successfully achieved and at a high scientific level. The results are formulated in 12 conclusions, which fully reflect the inferences of the studies. 9 contributions have also been formulated, which prove the scientific value of the dissertation work. Briefly presented, these are:

- An analytical method "Water. Determination of microcystin content by HPLC" has been developed and validated, introduced as an in-laboratory testing methodology and included in the scope of accreditation of the "ZDRAVE" Testing Center at the NCPHA. The method can be used to control the content of microcystin-LR, in accordance with Directive (EU) 2020/2184 and Regulation No. 9/2001 on the quality of water intended for drinking and domestic purposes.
- For the first time in Bulgaria, a systematic study of the seasonal dynamics (spring-summer-autumn) of microcystins and essential nutrients in water bodies used for drinking water supply in the period 2023–2025 has been carried out

- The dissertation provides a scientific basis for the introduction of monitoring of microcystin–LR in accordance with the requirements of Directive (EU) 2020/2184, including the concomitant homologues microcystin–YR and –RR for a more complete risk assessment.
- The information generated can be used in developing risk assessment and quality management programs for surface waters intended for drinking purposes.

In connection with the dissertation work, the doctoral student has presented three publications in which she is the first author, as well as participation in 9 scientific forums in which she presented reports and posters on issues related to the topic of the dissertation.

The overall impression of the scientific work, with the accompanying publications and participation in scientific conferences, is positive. The research are planned correctly and implemented qualitatively, there is highly qualified and precise experimental work, good scientific summaries

Based on the above, I give a positive assessment to the submitted to me for an opinion dissertation on the topic "Hygienic and analytical aspects in the study of the seasonal dynamics of microcystins in surface waters for drinking purposes". The scientific work meets the requirements of the Law on the State of the Republic of Bulgaria and the Regulations on the terms and conditions for acquiring scientific degrees and occupying academic positions at the National Center for Public Health and Analysis, therefore I propose to the esteemed members of the scientific jury to positively evaluate the work and support the awarding of the scientific and educational degree "Doctor" to Chief Assistant Professor Maria Angelova Mitreva in professional field 7.1 Medicine, scientific specialty "Hygiene" for the needs of the Department "Chemical Factors", Directorate "Analytical and Laboratory Activities" at the National Center for Public Health and Analysis.

Sofia, 16.04.2026 г.

Associate Professor Vessela Georgieva, PhD

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